Biology as a **Tool** for Synthetic Chemistry

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Denmark Group Meeting

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You Need 250 mg of Complex Molecule ***Xity-9er*** ASAP
What are Your Options?

	Pro's	Con's
▪ Biology ○ Kill (Harvest); ○ Isolation	Fast and Easy (1 day) Required tools (Jug, knife, sep-funnel Column, etc)	Potentially non-existant Probably limited availability Death of something
▪ Biology ○ Genetics • Directed Evolution (then isolation)	Good precedent Potentially fast (<1 yr) Potential to discover Something new exists	Limited by Organism lifespan. Requires more knowledge. Specialized tools.
▪ Chemistry ○ Synthetic Organic • Total Synthesis	~~Excellent precedent~~ Will work Potential to discover Something new is high	Requires lots of knowledge Requires lots of skill Requires lots of tools

A useful tidbit: Bacteria make up ~50% of the earth's biomass
animals make up ~0.1%

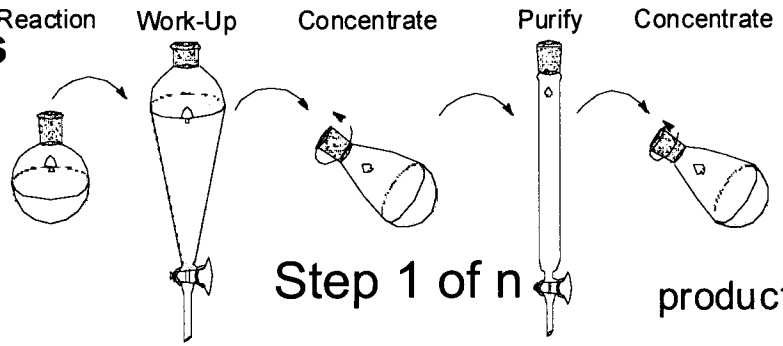
Despite the formalities and definitions, There are a Continuum of Possibilities and any Combination of these may be the *optimum* path to success.

Options in Chemical Pathway Design

space

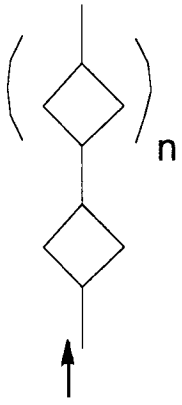
time

Option I: **different times**
Reaction occur in a vessel, but not all at the same time



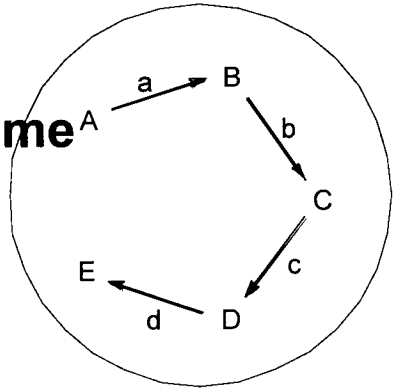
Tried and true
1-3 Rx/Day
Must rest

Option II: **different spaces**
Reactions occur in a single vessel at the same time but in different space



New and different
n Rx/Day
Never stops
(but could)

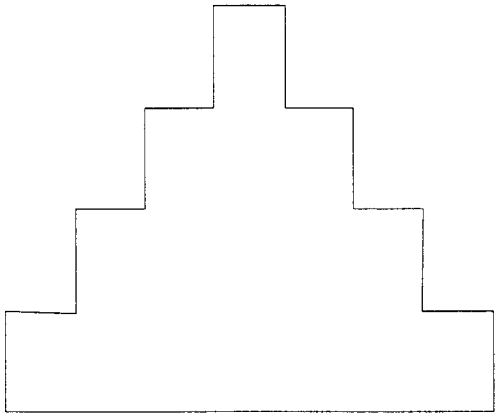
Option III: **same space, same time**
Reactions occur in a single vessel at the same time



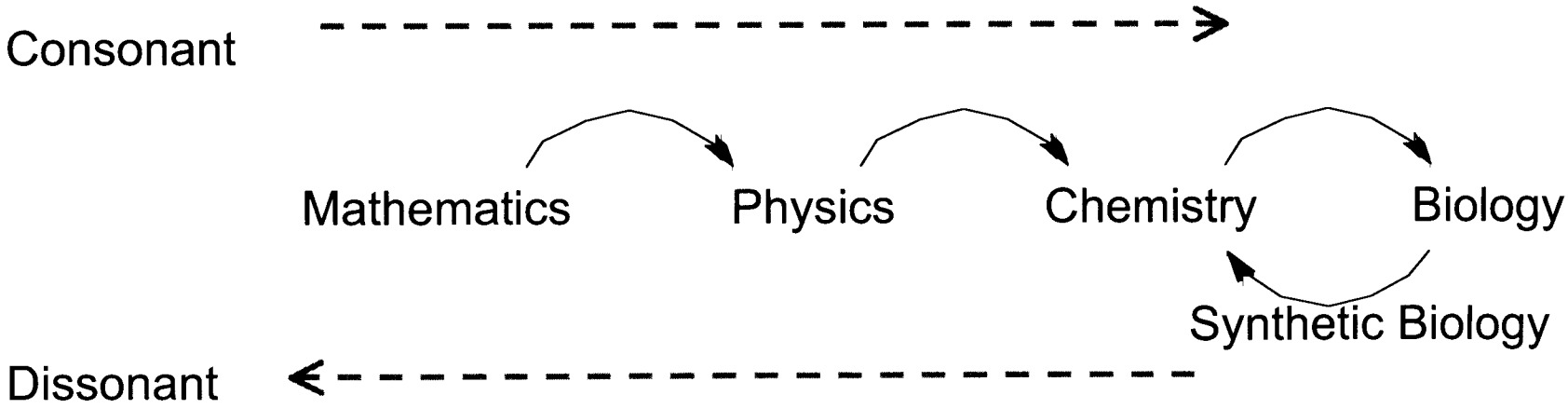
Tested by Life
~500 Rx/Day
E. coli
Stop = death

In Options II and III, once you are happy with the result, you quit.

Umpolung Science



Biology	Math + Physics + Chemistry + Life
Chemistry	Math + Physics applied to physical processes and change
Physics	Math applied to mass, energy and movement
Mathematics	Pure numbers: Logic Fundamental Concepts



Developing the Tools

- 500 BC – 500 AD
  - Botany, Zoology and dissection
  - Figure out "how things work" by inspecti

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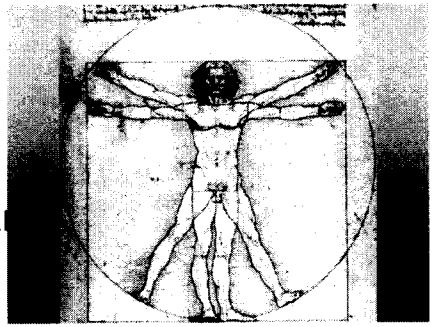
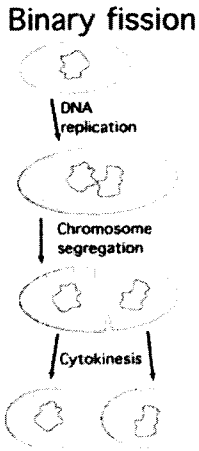
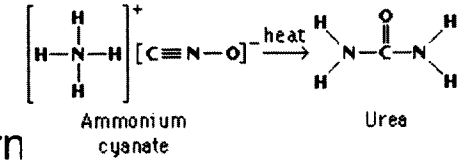
- ~500 – 1500 century AD
  - Middle Ages, scientific hietus,

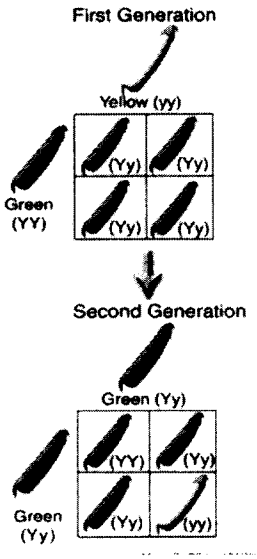


- 1500 – 1700: Naturalists and Observation
  - Classification and the microscope - Leeuwenhoek
  - animalcules and wee beasties (small life, short lifespan)
  - "Directed Evolution" exists but is not understood (breeding pla



- 1700 – 1850
  - Describe living things Phenotypically
  - Organize and Quantify (Antoine-Laurent de Lavoisier) Life
    - Conservation of Mass, Stoichiometry, Thermodynamics
    - Birth of Modern Chemistry
  - Photosynthesis discovered
  - Atomic Theory (1808)
  - Vitalism trashed: Friedrich Wöhler – (1828)

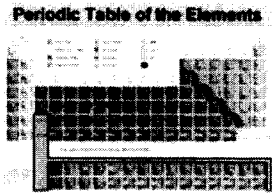




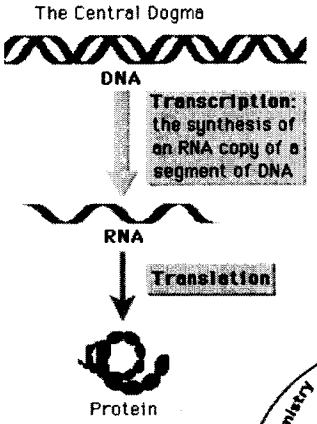
- 1850's Genetics and Evolution are born
  - Phenotypes can be ***Rationalized*** statistically (Genetics: Mendel)
  - ***And*** the average phenotype of a population can change (Evolution: Darwin)
  - Chemical Structure proof – Keküle - benzene

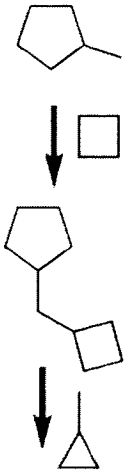
Genetics, Molecular Biology and Organic Chemistry

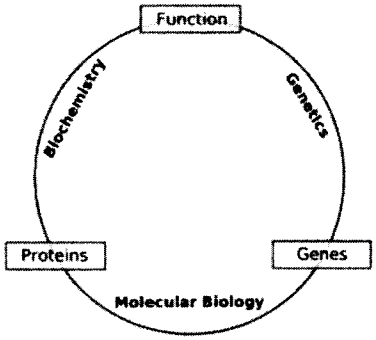
- 1850-1900
  - Rediscovery of mendel
  - Cell theory, embryology and germ theory
  - Periodic table – Mendeleyev
  - The electon – JJ Thompson



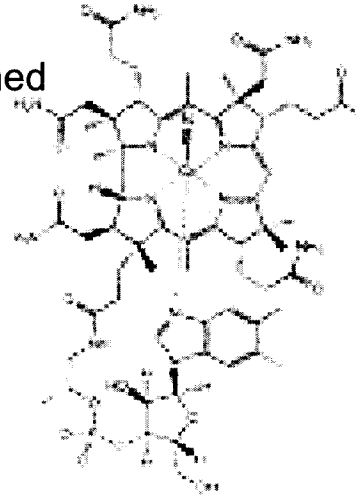
- 1900 – 1950
  - Molecular Biology becomes a field of study
  - Genes are on Chromosomes
  - Lewis Dot Structures
  - NMR is born (Purcell and Bloch)
  - Physical organic chemistry and reactive intermediates
  - Total synthesis if commerciallized (camphor)

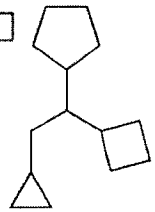






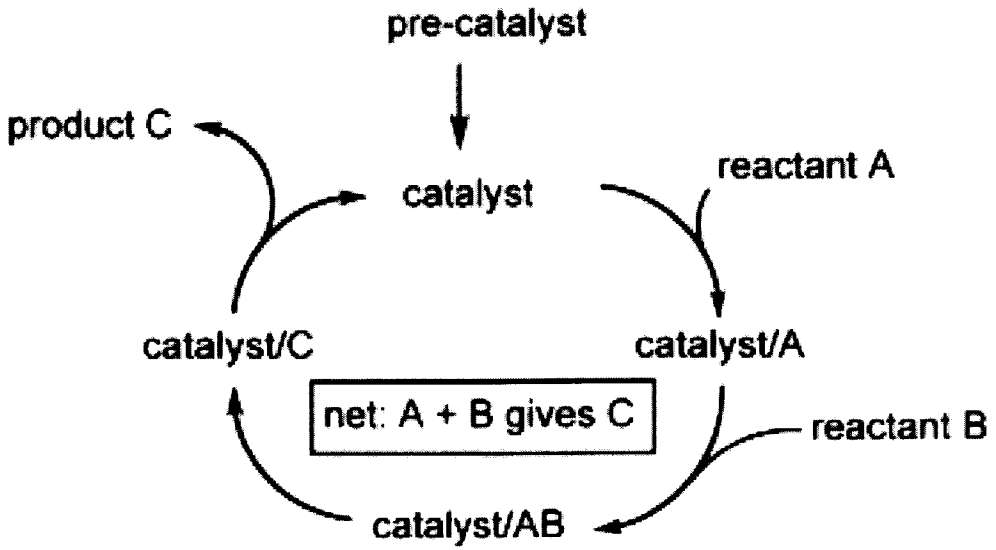
- 1950 – 1975
  - Intellectual core of Molecular Biology established
  - 1953 DNA structure solved
  - Learning AT and GC rules and sequencing
  - DNA to RNA to Protein dogma established
  - Genetics, molecular biology, cell biology
  - Primary and Secondary metabolic “maps” (eg. Terpenes)
  - Begin to “perturb” with molecules
  - We can make anything: Vitamin B12

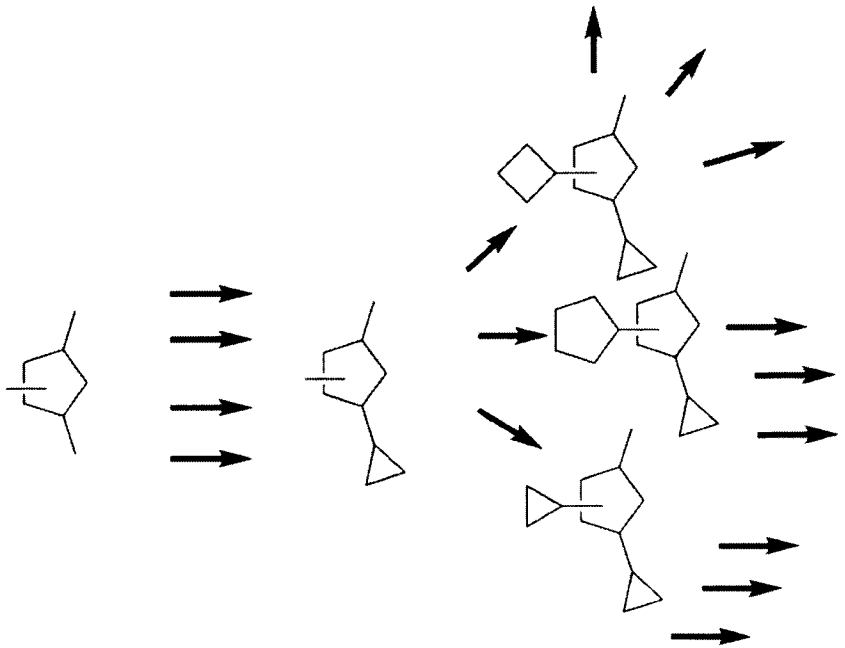




“Current” Status of Molecular Biology

- 1975 – today:
  - Collection of DATA
  - Sequence Bacteria, yeast, worm, fly, human…
  - Molecular biology, chemical biology, tools, tools, tools…
  - “omics” Huge amounts of data
  - Retrosynthetic Analysis
  - Many successful drugs developed
  - Combinatorial and Diversity Oriented synthesis born
  - Many types of Catalysis



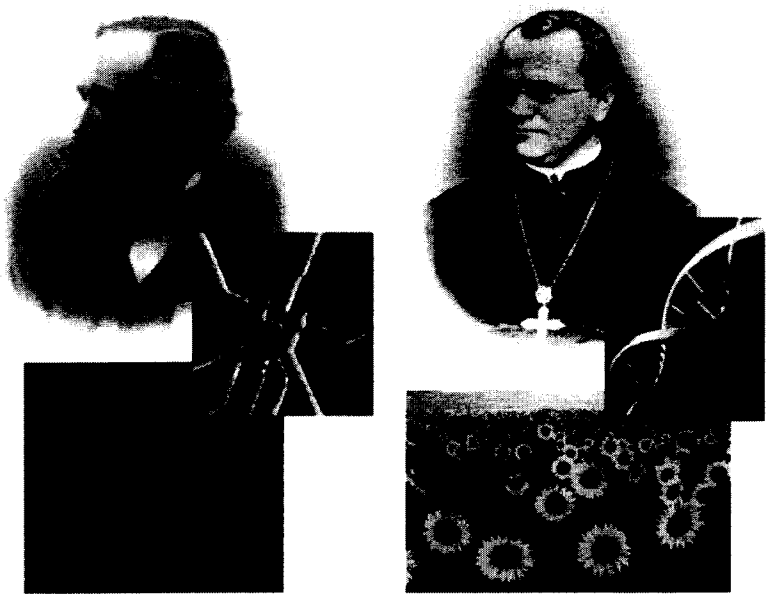


“Synthetic Biology”

In 1974, the Polish geneticist **Waclaw Szybalski** introduced the term "synthetic biology” in answer to the question "what next?".

His response; “Up to now we are working on the descriptive phase of molecular biology. But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field with the unlimited expansion potential and hardly any limitations to building "new better control circuits" and ..... finally other "synthetic"organisms, like a "new better mouse". ... I am not concerned that we will run out exciting and novel ideas, ... in the synthetic biology, in general.

James Clark Maxwell Gregor Mendel



In other words: “Like synthetic chemists, synthetic biologists are target-oriented.”
-Laura Kiessling

Today’s Talk: Using the concept of “Synthetic Biolc as a tool for Synthetic Organic Chemistry.

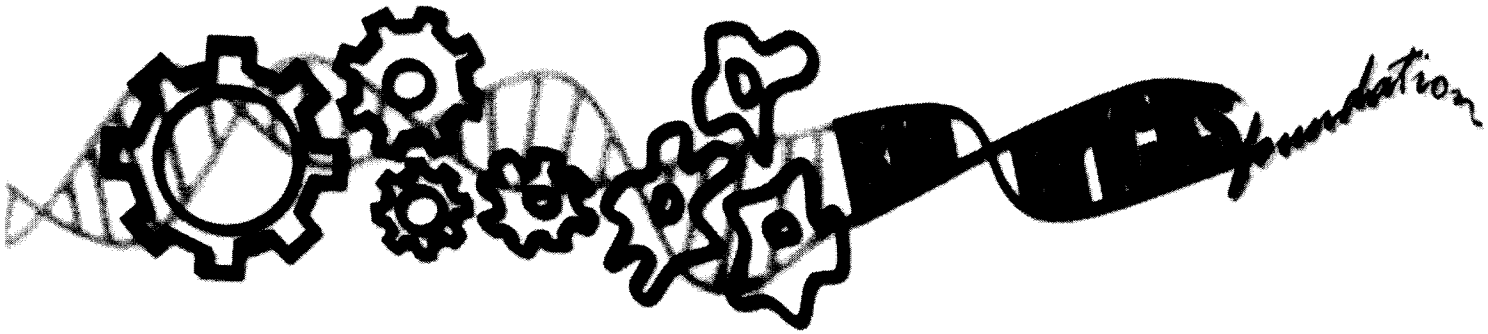
An Engineer's Impression of Life

Systems	Potentially many devices	incorporates "Life" self-maintaing synthetic pathway(s)
Devices	A few parts linked together	Synthetic pathway
Parts:	Proteins, mRNA, small molecules, etc	Catalyst
Storable Information	DNA	Genes and Gene Clusters

The Major Divisions

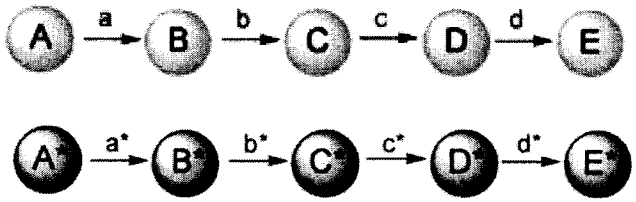
- Mutational Biosynthesis
  - Change (mutate or rearrange) existing Pathways
  - Success found in Primary metabolic pathways
  - Success found in polyketides, terpenes and nonribosomal peptide synthetases (secondary)
- Synthetic Biology
  - Engineering using the machinery of a cell.
  - Combine "parts" to make devices: Sensors, signals, logic gates, computers, synthetic pathways…
- Genetic Engineering
  - Modify, Design, build, and standardize the genetic parts (genes)

- NOTE: An integral (and vital) field of study is developing suitable host species and strains. While interesting, this topic is outside the scope of the talk.

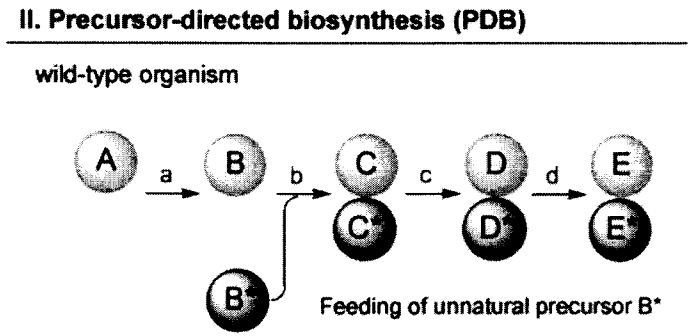


Common Methods Employed

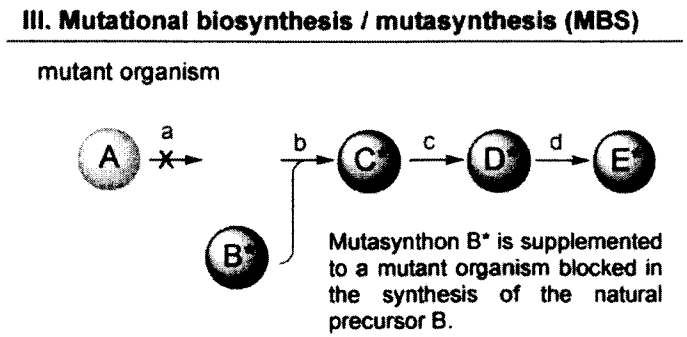
1. Biosynthesis of natural products



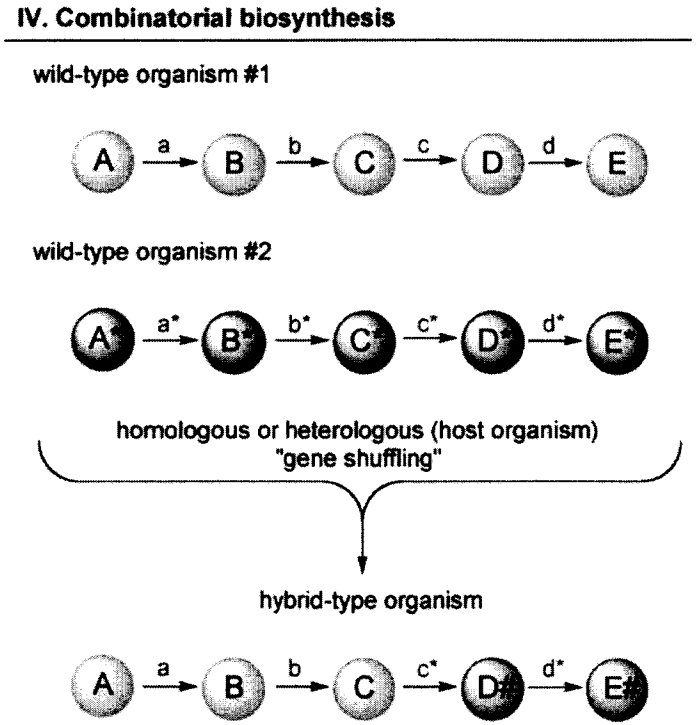
2. Precursor-directed biosynthesis



3. Mutational Biosynthesis

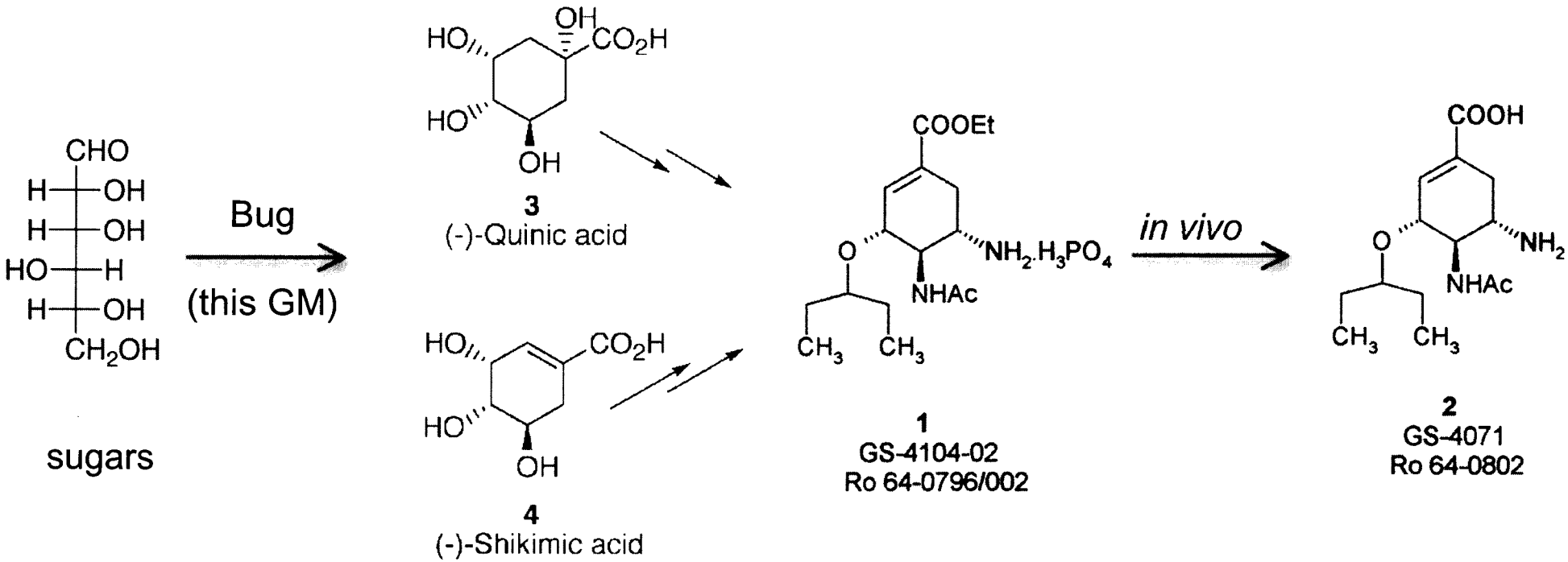


4. Combinatorial biosynthesis



If we place the emphasis on "Pathway Design", then Any Combination of these + flow + batch processes could be optimal.

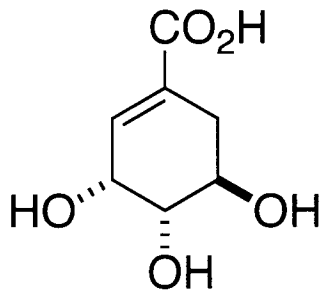
Shikimic Acid & Tamiflu (A Brief Reminder)



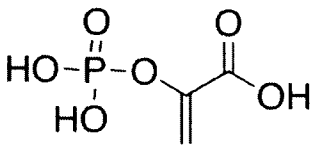
Previous GM

Shikimic Acid

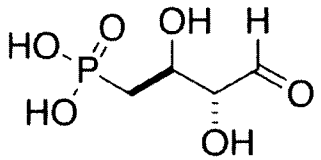
- Propose a Synthesis:



Some Starting Materials (optional)

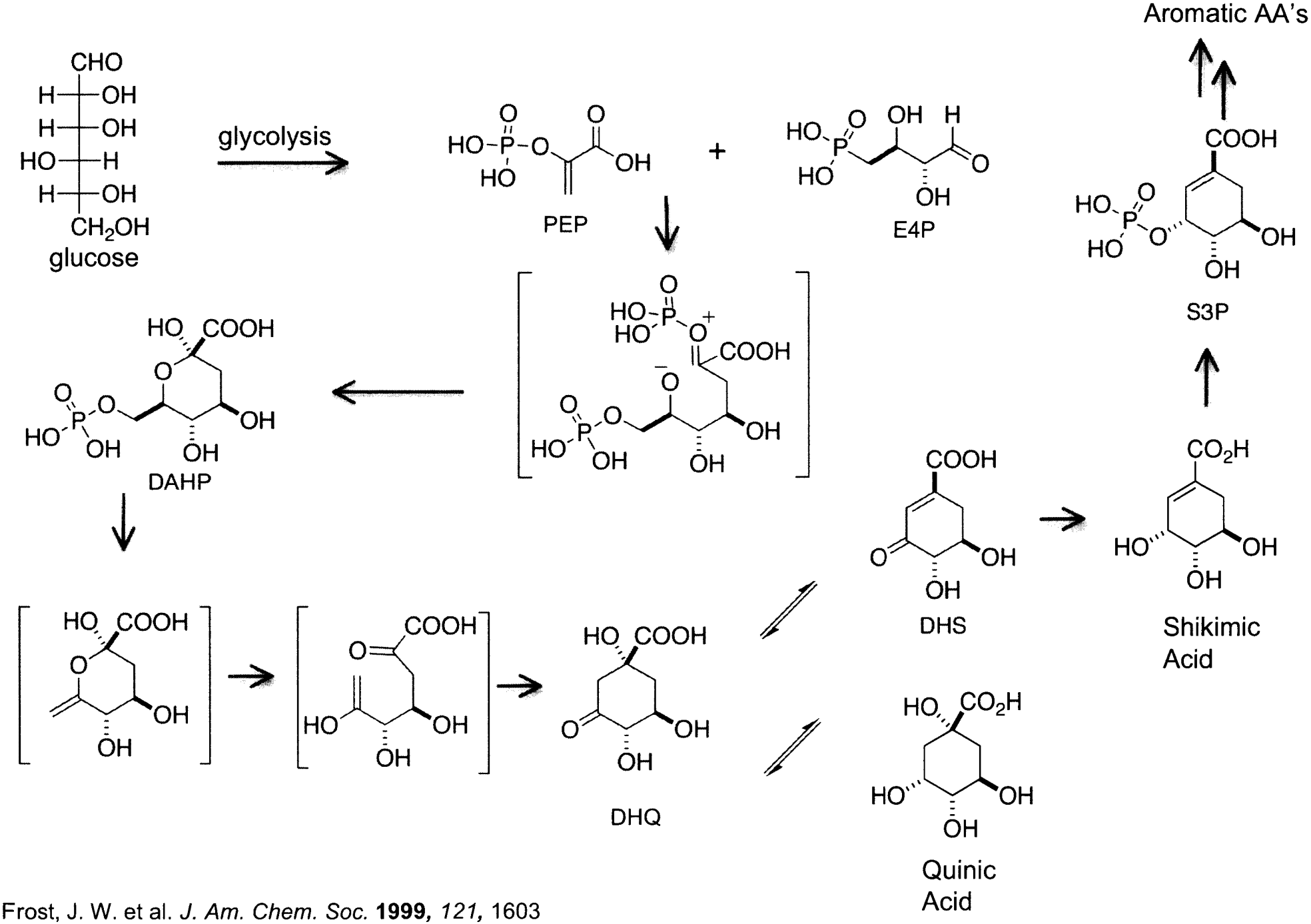


PEP



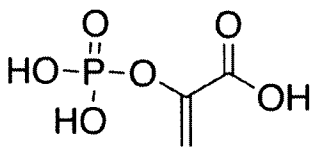
E4P

ake My Starting Material *E. coli*: Shikimic Acid



Frost, J. W. et al. *J. Am. Chem. Soc.* **1999,** *121,* 1603

Optimized Biosynthesis: Shikimic Acid



PEP

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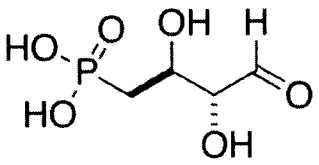

E4P

transketolase

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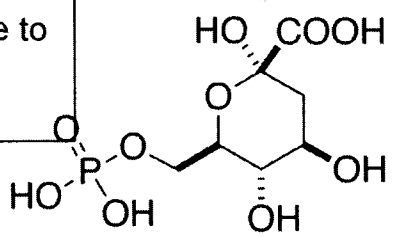
Pentose Phosphate Pathway

DAHP synthase

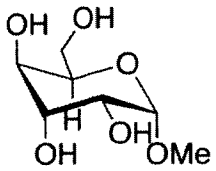
Inserted a copy insensitive to Feedback inhibition From Arom. AA's



DAHP

Operational Optimization

1. Increased glucose concentration
2. Addition of glucose mimic:
3. Collection of acids by ion exchange
4. Removal of quinic acid by Recrystallization



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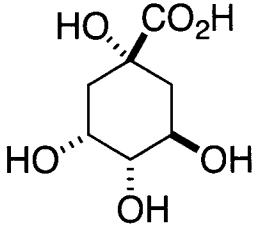
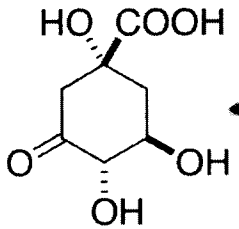
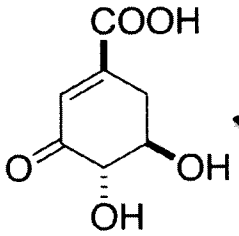
DHQ synthase

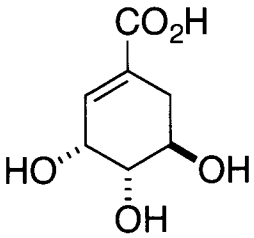




DHQ

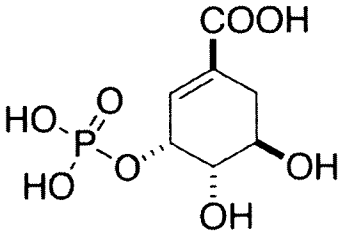


DHS



Shikimate Kinase

Knocked Out

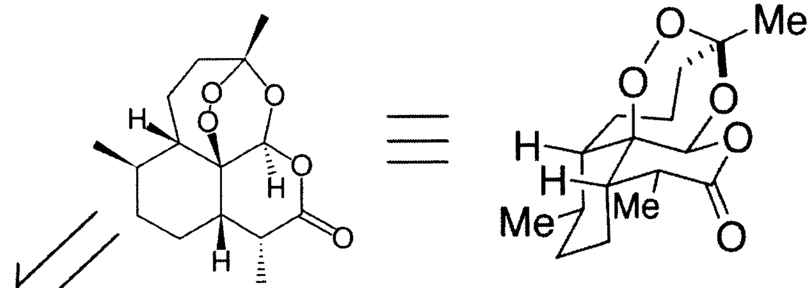


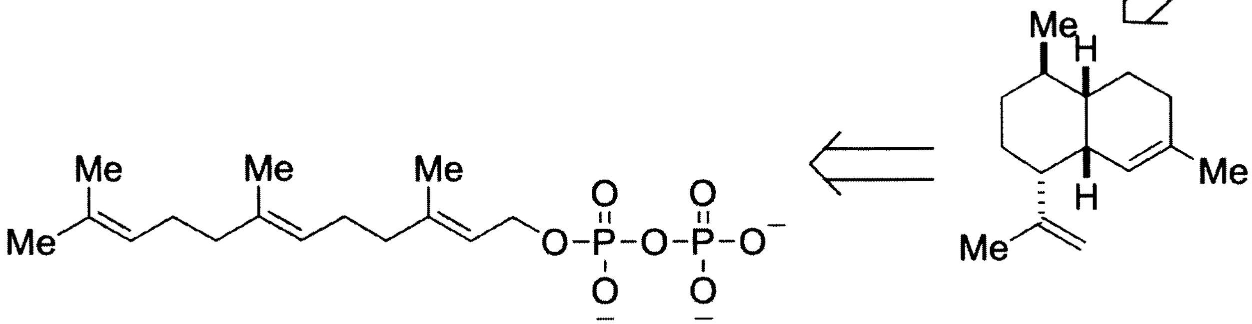
S3P

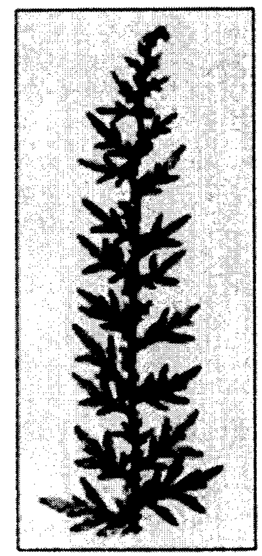
Shikimate Dehydrogenase
Extra copy to minimize Feedback inhibition

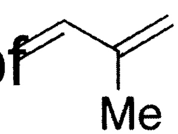
Final result: ~40 g/L in ~35 h

Artemisinin: An Example of a Secondary Metabolite



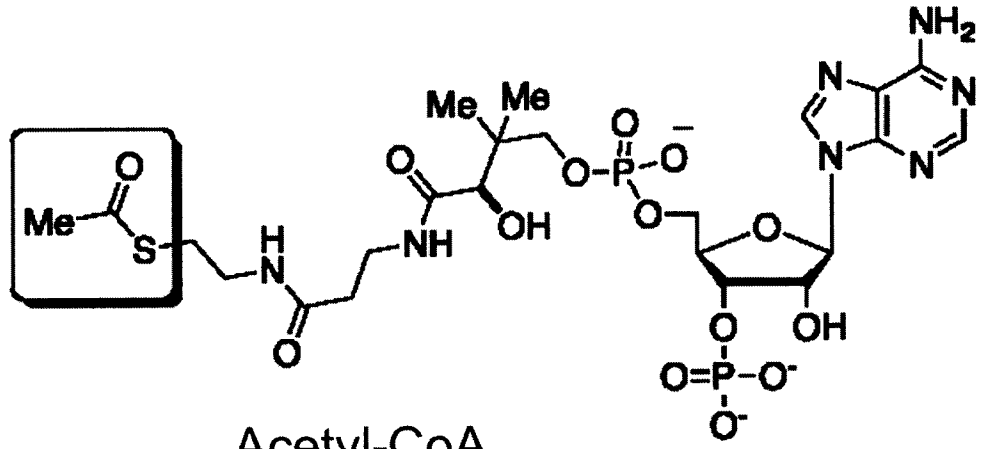




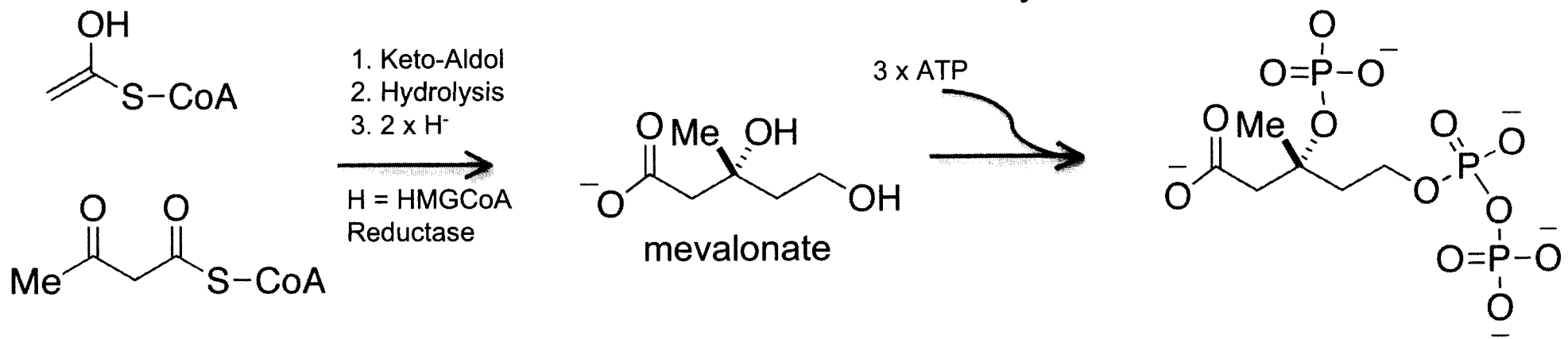
- Isolated from *Artemisia annua* (~270 mg/250 g)
- Strong anti-malarial activity (too expensive)
- Polyoxygenated: lactone and endoperoxide
- 1 of ~55,000 known terpenes: polymer of 

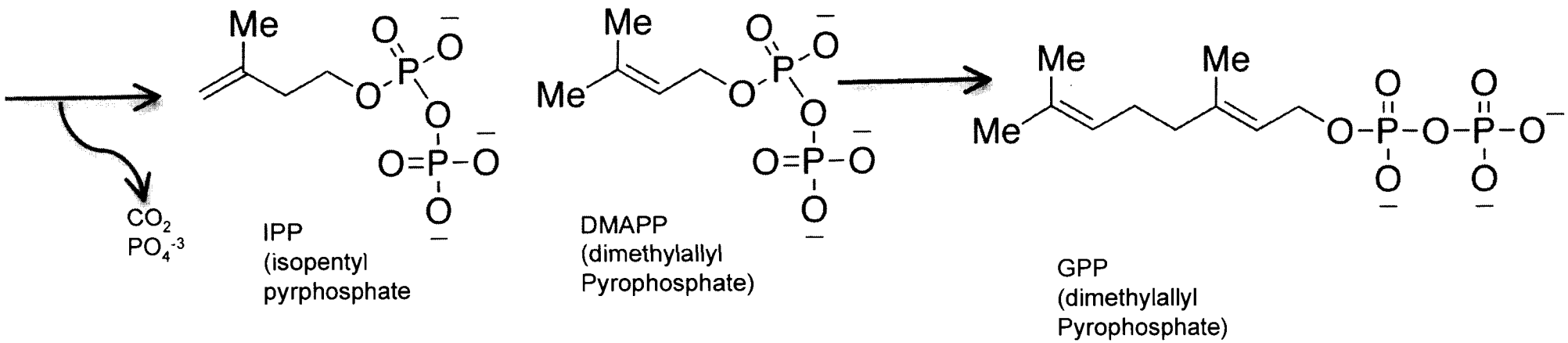
- A sesquiterpene (3 isoprene units → FPP derived)
- Oxidized after cyclization

Mevalonate Dependent Terpene biosynthesis

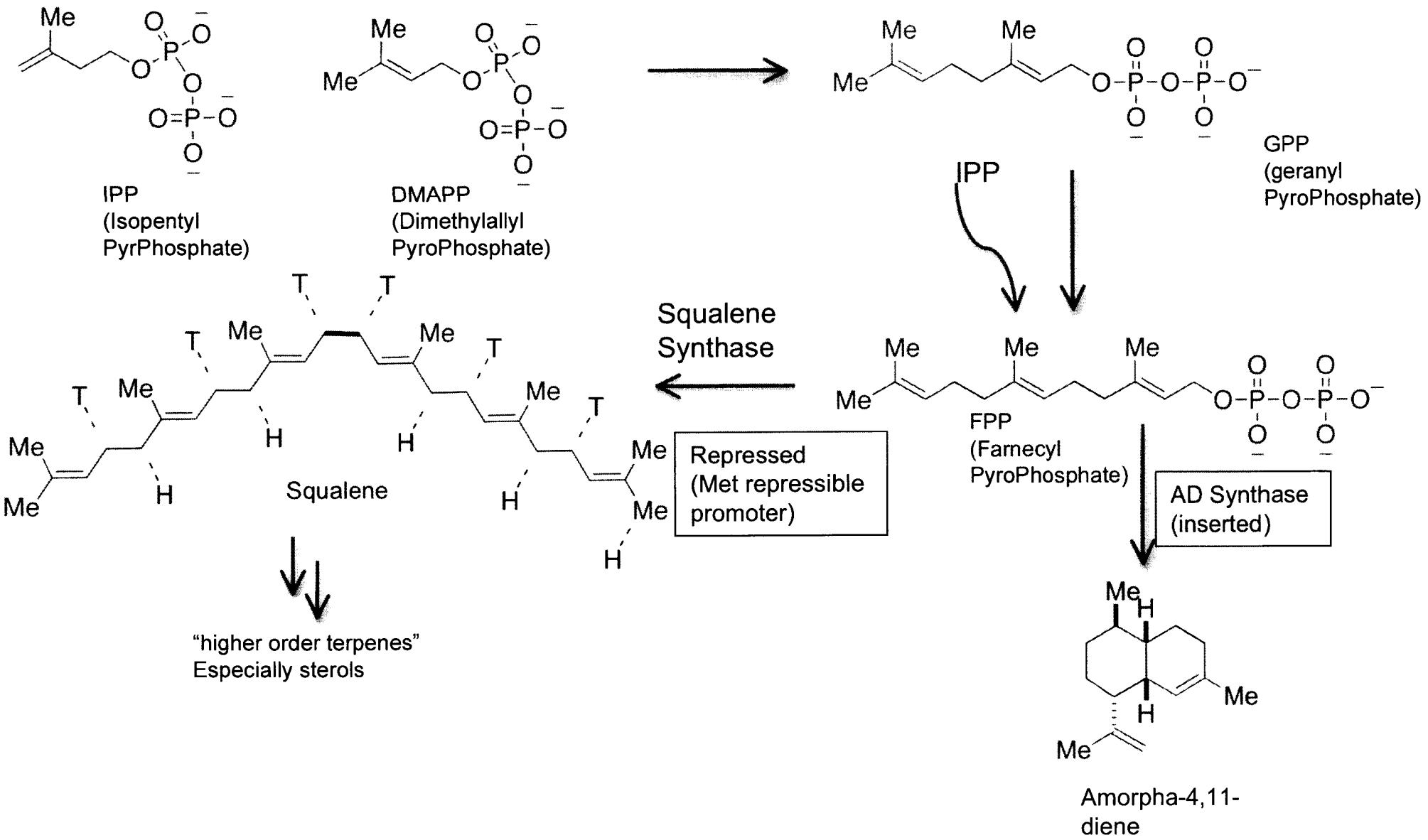


Acetyl-CoA

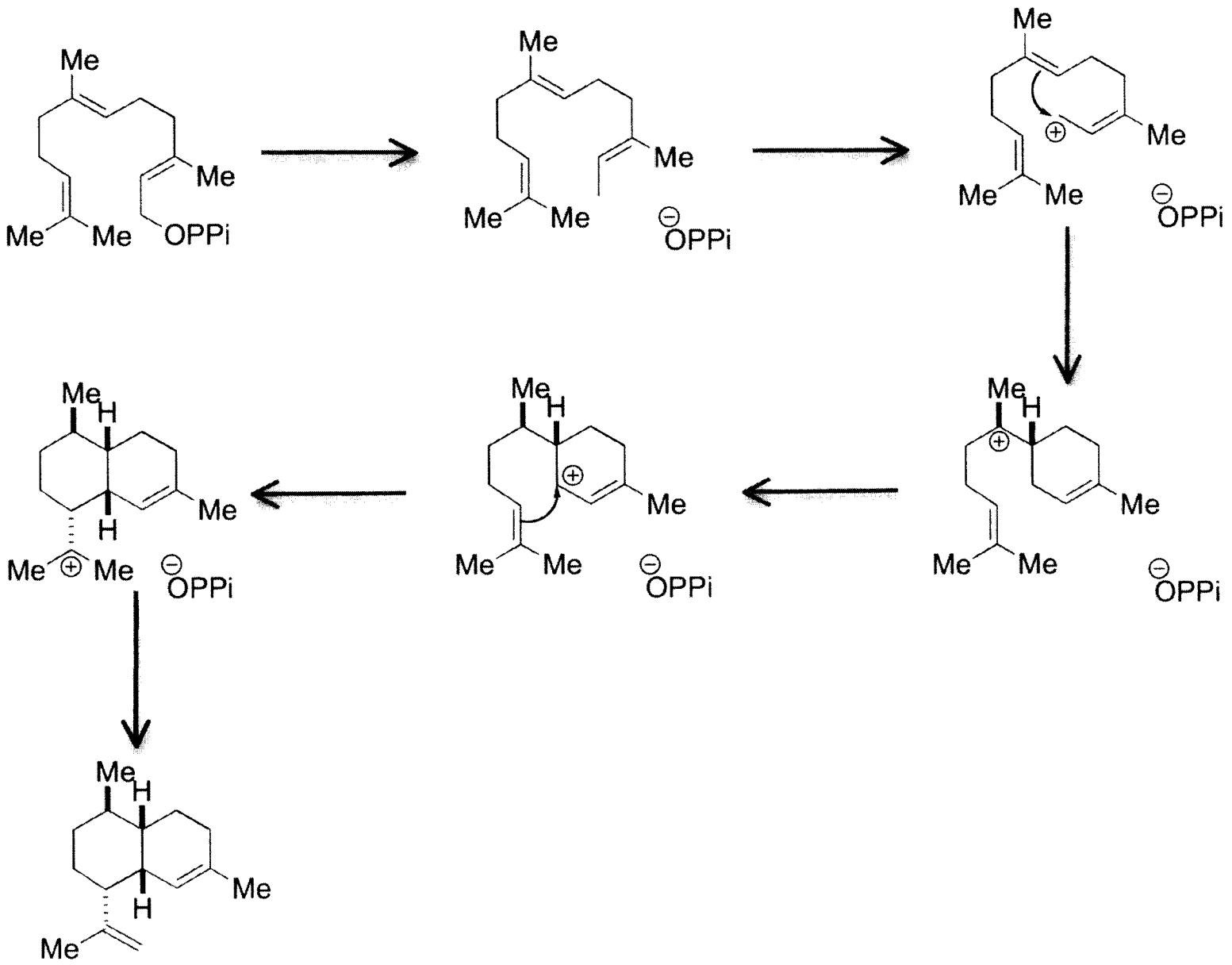




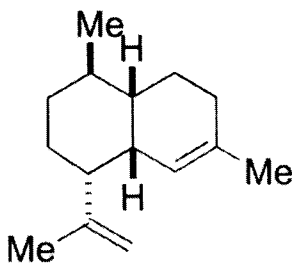
Mev. Dep. Pathway Optimized for Artemisinin Synthesis



Proposed Mechanism



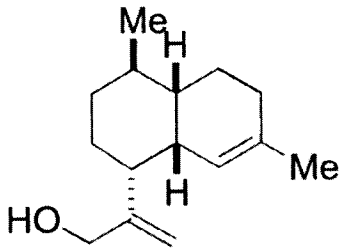
Optimized End Game for Artimisinin



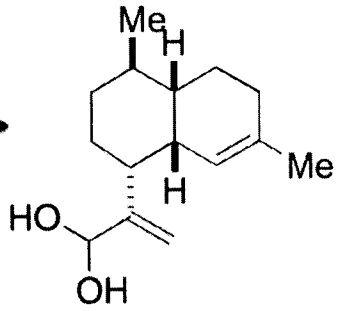
Amorpha-4,11-diene

Inserted

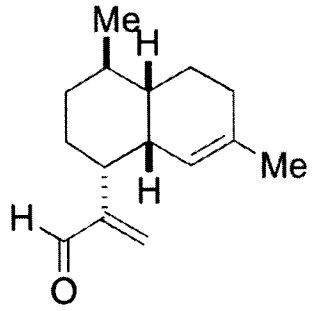
P450 and CPR



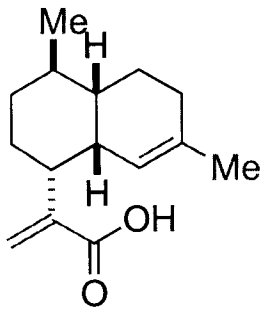
P450 and CPR



Identified a cytochrome P450 monooxygenase gene and its redox partner (cytochrome P450 oxidoreductase – CPR) by sequence analogy. Cloned it and inserted into yeast genome.



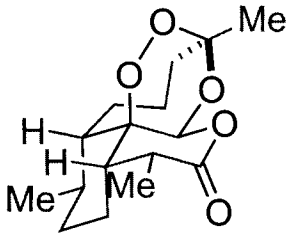
P450 and CPR



~115 mg/L
(similar to *A. annua)*
Production time decreased from ~3 months to 4 days

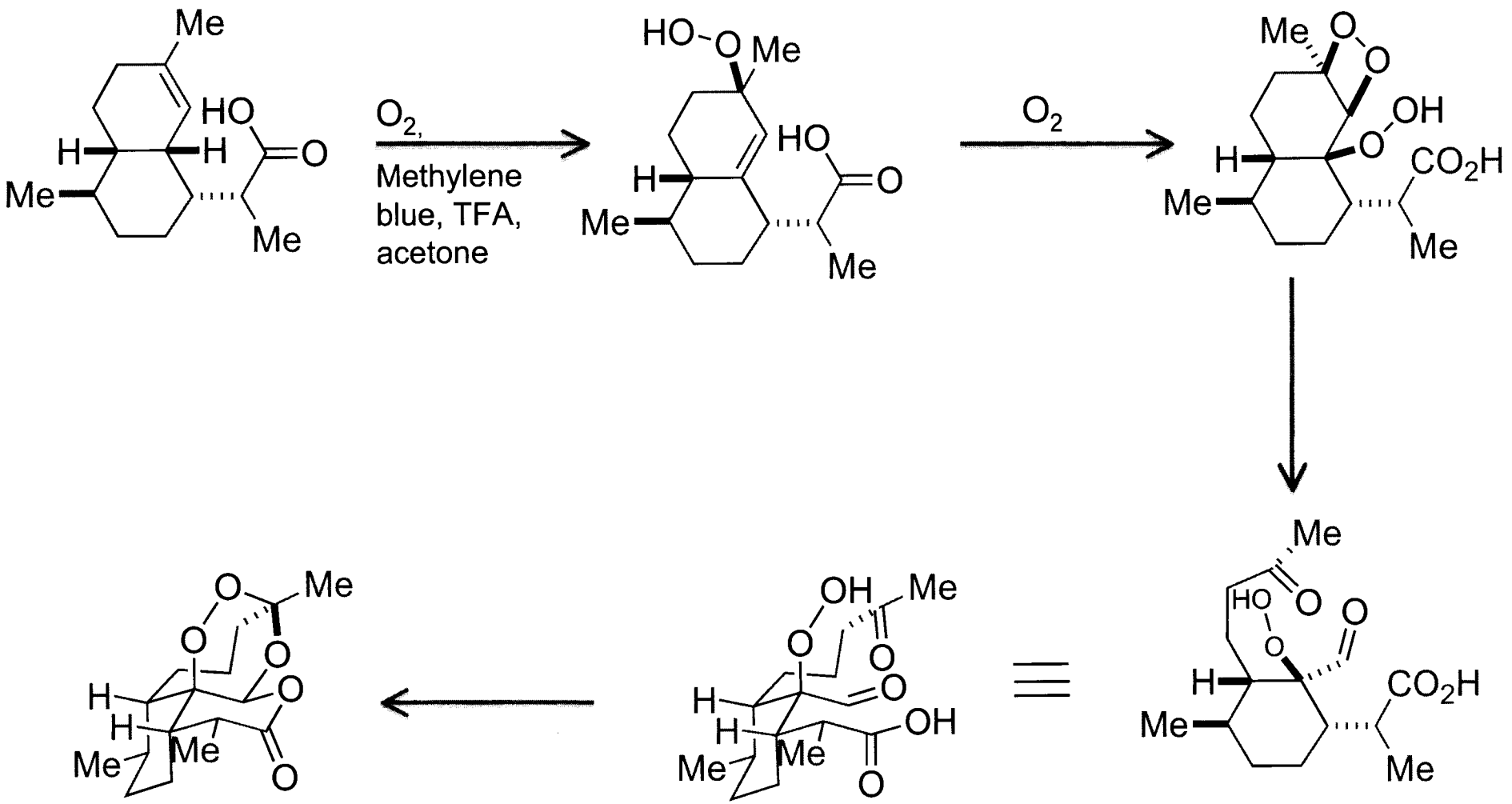
Unknown Biochemistry

Organic Synthesis



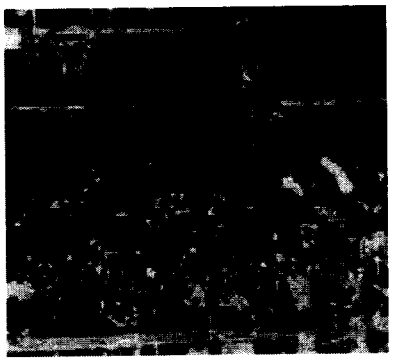
Reduced to **~12potential genes**……but no data yet

Artemisinin from Artimisinic Acid



Conclusions and Future Directions

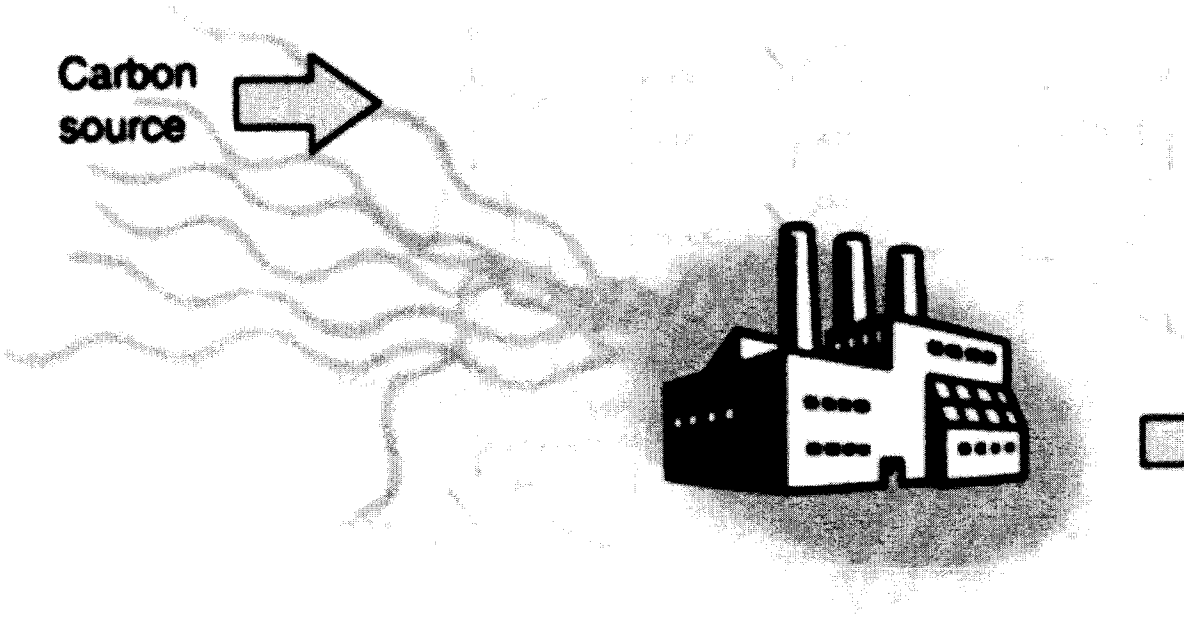
- Conclusion: It works
  - Where does the field sit?
  - What is there to do?



- My Opinion: The ideal situation, for the synthetic organic chemist, would be a simple, borderless interphase between our science, synthetic biology and metabolomics.
  - Give synthetic chemists a new "vantage point."
  - Provide "synthetic biologists" with a never-ending list of targets.

Organic Chemistry (masters of molecule making)	"Omics" (massive piles of unsifted biological data)	Synthetic Biology (master of "pathway making")

Future Directions



Starting Material
Intermediates
Entire Drugs
Libraries
Integrated flow
pathways?

In essense “never-stopping” synthetic machines…..powered by Life.

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